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(54) REGULATION OF QUINOLATE PHOSPHORIBOSYL TRANSFERASE EXPRESSION

REGULATION DER EXPRESSION VON CHINOLATPHOSPHORIBOSYLTRANSFERASE

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of nicotine in leaves of the tobacco plant by growing a tobacco plant with cells that comprise an exogenous DNA sequence, where a transcribed strand of the exogenous DNA sequence is complementary to endogenous quinolate phosphoribosyl transferase messenger RNA in the cells.

[0011] A further aspect of the present invention is a method of making a transgenic plant cell having increased quinolate phosphoribosyl transferase (QPRTase) expression, by transforming a plant cell known to express quinolate phosphoribosyl transferase with an exogenous DNA construct which comprises a DNA sequence encoding quinolate phosphoribosyl transferase.

[0012] A further aspect of the present invention is a transgenic *Nicotiana* plant having increased quinolate phosphoribosyl transferase (QPRTase) expression, where cells of the transgenic plant comprise an exogenous DNA sequence encoding a plant quinolate phosphoribosyl transferase.

[0013] A further aspect of the present invention is a method for increasing expression of a quinolate phosphoribosyl transferase gene in a plant cell, by growing a plant cell transformed to contain exogenous DNA encoding quinolate phosphoribosyl transferase.

[0014] A further aspect of the present invention is a method of producing a tobacco plant having increased levels of nicotine in the leaves, by growing a tobacco plant having cells that contain an exogenous DNA sequence that encodes quinolate phosphoribosyl transferase functional in the cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015]

Figure 1 shows the biosynthetic pathway leading to nicotine. Enzyme activities known to be regulated by *Nic1* and *Nic2* are QPRTase (quinolate phosphoribosyl transferase) and PMTase (putrescence methyl-transferase).

Figure 2A provides the nucleic acid sequence of *NtQPT1* cDNA (**SEQ ID NO:1**), with the coding sequence (SEQ ID NO:3) shown in capital letters.

Figure 2B provides the deduced amino acid sequence (**SEQ ID NO:2**) of the tobacco QPRTase encoded by *NtQPT1* cDNA.

Figure 3 aligns the deduced NtQPT1 amino acid sequence and related sequences of *Rhodospirillum rubrum, Mycobacterium lepre, Salmonella typhimurium, Escherichia coli,* human, and *Saccharomyces cerevisiae.*

Figure 4 shows the results of complementation of an *Escherichia coli* mutant lacking quinolate phosphoribosyl transferase (TH265) with *NtQPT1* cDNA. Cells were transformed with an expression vector carrying *NtQPT1*; growth of transformed TH265 cells expressing *NtQPT1* on minimal medium lacking nicotinic acid demonstrated that *NtQPT1* encodes QPRTase.

Figure 5 compares nicotine levels and the relative steady-state *NtQTP1* mRNA levels in *Nic1* and *Nic2* tobacco mutants: wild-type Burley 21 (*Nic1/Nic1 Nic2/Nic2*); *Nic1*⁻ Burley 21 (*nic1/nic1 Nic2/Nic2*); *Nic2*⁻ Burley 21 (*Nic1/Nic1 nic2/nic2);* and *Nic1*⁻*Nic2*⁻ Burley 21 (*nic1/nic1 nic2/nic2).* Solid bars indicate mRNA transcript levels; hatched bars indicate nicotine levels.

Figure 6 charts the relative levels of *NtQPT1* mRNA over time in topped tobacco plants compared to non-topped control plants. Solid bars indicate mRNA transcript levels; hatched bars indicate nicotine levels.

DETAILED DESCRIPTION OF THE INVENTION

[0016] Nicotine is produced in tobacco plants by the condensation of nicotinic acid and 4-methylaminobutanal. The biosynthetic pathway resulting in nicotine production is illustrated in **Figure 1.** Two regulatory loci (*Nic1* and *Nic2*) act as co-dominant regulators of nicotine production. Enzyme analyses of roots of single and double *Nic* mutants show that the activities of two enzymes, quinolate phosphoribosyl transferase (QPRTase) and putrescence methyl transferase (PMTase), are directly proportional to levels of nicotine biosynthesis. A comparison of enzyme activity in tobacco tissues (root and callus) with different capacities for nicotine synthesis shows that QPRTase activity is strictly correlated with nicotine content (Wagner and Wagner, *Planta* 165:532 (1985)). Saunders and Bush *(Plant Physiol* 64:236 (1979) showed that the level of QPRTase in the roots of low nicotine mutants is proportional to the levels of nicotine in the leaves.

[0017] The present invention encompasses a novel cDNA sequence (**SEQ ID NO:1**) encoding a plant quinolate phosphoribosyl transferase (QPRTase) of **SEQ ID NO:2**. As QPRTase activity is strictly correlated with nicotine content, construction of transgenic tobacco plants in which QPRTase levels are lowered in the plant roots (compared to levels in wild-type plants) result in plants having reduced levels of nicotine in the leaves. The present invention provides methods and nucleic acid constructs for producing such transgenic plants, as well as such transgenic plants. Such methods include the expression of antisense *NtQPT1* RNA, which lowers the amount of QPRTase in tobacco roots. Nicotine has additionally been found in non-tobacco species and families of plants, though the amount present is

into a cell (or the cell's ancestor) through the efforts of humans. Such heterologous DNA may be a copy of a sequence which is naturally found in the cell being transformed, or fragments thereof.

[0028] To produce a tobacco plant having decreased QPRTase levels, and thus lower nicotine content, than an untransformed control tobacco plant, a tobacco cell may be transformed with an exogenous QPRT antisense transcriptional unit comprising a partial QPRT cDNA sequence, a full-length QPRT cDNA sequence, a partial QPRT chromosomal sequence, or a full-length QPRT chromosomal sequence, in the antisense orientation with appropriate operably linked regulatory sequences. Appropriate regulatory sequences include a transcription initiation sequence ("promoter") operable in the plant being transformed, and a polyadenylation/transcription termination sequence. Standard techniques, such as restriction mapping, Southern blot hybridization, and nucleotide sequence analysis, are then employed to identify clones bearing QPRTase sequences in the antisense orientation, operably linked to the regulatory sequences. Tobacco plants are then regenerated from successfully transformed cells. It is most preferred that the antisense sequence utilized be complementary to the endogenous sequence, however, minor variations in the exogenous and endogenous sequences may be tolerated. It is preferred that the antisense DNA sequence be of sufficient sequence similarity that it is capable of binding to the endogenous sequence in the cell to be regulated, under stringent conditions as described below.

[0029] Antisense technology has been employed in several laboratories to create transgenic plants characterized by lower than normal amounts of specific enzymes. For example, plants with lowered levels of chalcone synthase, an enzyme of a flower pigment biosynthetic pathway, have been produced by inserting a chalcone synthase antisense gene into the genome of tobacco and petunia. These transgenic tobacco and petunia plants produce flowers with lighter than normal coloration (Van der Krol et al., "An Anti-Sense Chalcone Synthase Gene in Transgenic Plants Inhibits Flower Pigmentation", *Nature*, 333, pp. 866-69 (1988)). Antisense RNA technology has also been successfully employed to inhibit production of the enzyme polygalacturonase in tomatoes (Smith et al., "Antisense RNA Inhibition of Polygalacturonase Gene Expression in Transgenic Tomatoes", *Nature*, 334, pp. 724-26 (1988); Sheehy et al., "Reduction of Polygalacturonase Activity in Tomato Fruit by Antisense RNA", *Proc. Natl. Acad Sci. USA*, 85, pp. 8805-09 (1988)), and the small subunit of the enzyme ribulose bisphosphate carboxylase in tobacco (Rodermel et al., "Nuclear-Organelle Interactions: Nuclear Antisense Gene Inhibits Ribulose Bisphosphate Carboxylase Enzyme Levels in Transformed Tobacco Plants", *Cell*, 55, pp. 673-81 (1988)). Alternatively, transgenic plants characterized by greater than normal amounts of a given enzyme may be created by transforming the plants with the gene for that enzyme in the sense (i.e., normal) orientation. Levels of nicotine in the transgenic tobacco plants of the present invention can be detected by standard nicotine assays. Transformed plants in which the level of QPRTase is reduced compared to untransformed control plants will accordingly have a reduced nicotine level compared to the control; transformed plants in which the level of QPRTase is increased compared to untransformed control plants will accordingly have an increased nicotine level compared to the control.

[0030] The heterologous sequence utilized in the antisense methods of the present invention may be selected so as to produce an RNA product complementary to the entire QPRTase mRNA sequence, or to a portion thereof. The sequence may be complementary to any contiguous sequence of the natural messenger RNA, that is, it may be complementary to the endogenous mRNA sequence proximal to the 5'-terminus or capping site, downstream from the capping site, between the capping site and the initiation codon and may cover all or only a portion of the non-coding region, may bridge the non-coding and coding region, be complementary to all or part of the coding region, complementary to the 3'-terminus of the coding region, or complementary to the 3'-untranslated region of the mRNA. Suitable antisense sequences may be from at least about 13 to about 15 nucleotides, at least about 16 to about 21 nucleotides, at least about 20 nucleotides, at least about 30 nucleotides, at least about 50 nucleotides, at least about 75 nucleotides, at least about 100 nucleotides, at least about 125 nucleotides, at least about 150 nucleotides, at least about 200 nucleotides, or more. In addition, the sequences may be extended or shortened on the 3' or 5' ends thereof.

[0031] The particular anti-sense sequence and the length of the anti-sense sequence will vary depending upon the degree of inhibition desired, the stability of the anti-sense sequence, and the like. One of skill in the art will be guided in the selection of appropriate QPRTase antisense sequences using techniques available in the art and the information provided herein. With reference to **Figure 2A** and **SEQ ID NO:1** herein, an oligonucleotide of the invention may be a continuous fragment of the QPRTase cDNA sequence in antisense orientation, of any length that is sufficient to achieve the desired effects when transformed into a recipient plant cell.

[0032] The present invention may also be used in methods of sense co-suppression of nicotine production. Sense DNAs employed in carrying out the present invention are of a length sufficient to, when expressed in a plant cell, suppress the native expression of the plant QPRTase protein as described herein in that plant cell. Such sense DNAs may be essentially an entire genomic or complementary DNA encoding the QPRTase enzyme, or a fragment thereof, with such fragments typically being at least 15 nucleotides in length. Methods of ascertaining the length of sense DNA that results in suppression of the expression of a native gene in a cell are available to those skilled in the art.

[0033] In an alternate embodiment of the present invention, *Nicotiana* plant cells are transformed with a DNA construct containing a DNA segment encoding an enzymatic RNA molecule (*i.e.*, a "ribozyme"), which enzymatic RNA

RNA or amino acid sequences which have slight and non-consequential sequence variations from the actual sequences disclosed and claimed herein are considered to be equivalent to the sequences of the present invention. In this regard, "slight and non-consequential sequence variations" mean that "similar" sequences (i.e., the sequences that have substantial sequence similarity with the DNA, RNA, or proteins disclosed and claimed herein) will be functionally equivalent to the sequences disclosed and claimed in the present invention. Functionally equivalent sequences will function in substantially the same manner to produce substantially the same compositions as the nucleic acid and amino acid compositions disclosed and claimed herein.

[0041] DNA sequences provided herein can be transformed into a variety of host cells. A variety of suitable host cells, having desirable growth and handling properties, are readily available in the art.

[0042] Use of the phrase "isolated" or "substantially pure" in the present specification and claims as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have been separated from their *in vivo* cellular environments through the efforts of human beings.

[0043] As used herein, a "native DNA sequence" or "natural DNA sequence" means a DNA sequence which can be isolated from non-transgenic cells or tissue. Native DNA sequences are those which have not been artificially altered, such as by site-directed mutagenesis. Once native DNA sequences are identified, DNA molecules having native DNA sequences may be chemically synthesized or produced using recombinant DNA procedures as are known in the art. As used herein, a native plant DNA sequence is that which can be isolated from non-transgenic plant cells or tissue. As used herein, a native tobacco DNA sequence is that which can be isolated from non-transgenic tobacco cells or tissue.

[0044] DNA constructs, or "transcription cassettes," of the present invention include, 5' to 3' in the direction of transcription, a promoter as discussed herein, a DNA sequence as discussed herein operatively associated with the promoter, and, optionally, a termination sequence including stop signal for RNA polymerase and a polyadenylation signal for polyadenylase. All of these regulatory regions should be capable of operating in the cells of the tissue to be transformed. Any suitable termination signal may be employed in carrying out the present invention, examples thereof including, but not limited to, the nopaline synthase *(nos)* terminator, the octapine synthase (*ocs*) terminator, the CaMV terminator, or native termination signals derived from the same gene as the transcriptional initiation region or derived from a different gene. *See, e.g.,* Rezian et al. (1988) supra, and Rodermel et al. (1988), supra.

[0045] The term "operatively associated," as used herein, refers to DNA sequences on a single DNA molecule which are associated so that the function of one is affected by the other. Thus, a promoter is operatively associated with a DNA when it is capable of affecting the transcription of that DNA (i.e., the DNA is under the transcriptional control of the promoter). The promoter is said to be "upstream" from the DNA, which is in turn said to be "downstream" from the promoter.

[0046] The transcription cassette may be provided in a DNA construct which also has at least one replication system. For convenience, it is common to have a replication system functional in *Escherichia coli,* such as ColE1, pSC101, pACYC184, or the like. In this manner, at each stage after each manipulation, the resulting construct may be cloned, sequenced, and the correctness of the manipulation determined. In addition, or in place of the *E. coli* replication system, a broad host range replication system may be employed, such as the replication systems of the P-1 incompatibility plasmids, e.g., pRK290. In addition to the replication system, there will frequently be at least one marker present, which may be useful in one or more hosts, or different markers for individual hosts. That is, one marker may be employed for selection in a prokaryotic host, while another marker may be employed for selection in a eukaryotic host, particularly the plant host. The markers may be protection against a biocide, such as antibiotics, toxins, heavy metals, or the like; may provide complementation, by imparting prototrophy to an auxotrophic host; or may provide a visible phenotype through the production of a novel compound in the plant.

[0047] The various fragments comprising the various constructs, transcription cassettes, markers, and the like may be introduced consecutively by restriction enzyme cleavage of an appropriate replication system, and insertion of the particular construct or fragment into the available site. After ligation and cloning the DNA construct may be isolated for further manipulation. All of these techniques are amply exemplified in the literature as exemplified by J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2d Ed. 1989)(Cold Spring Harbor Laboratory).

[0048] Vectors which may be used to transform plant tissue with nucleic acid constructs of the present invention include both *Agrobacterium* vectors and ballistic vectors, as well as vectors suitable for DNA-mediated transformation.

[0049] The term 'promoter' refers to a region of a DNA sequence that incorporates the necessary signals for the efficient expression of a coding sequence. This may include sequences to which an RNA polymerase binds but is not limited to such sequences and may include regions to which other regulatory proteins bind together with regions involved in the control of protein translation and may include coding sequences.

[0050] Promoters employed in carrying out the present invention may be constitutively active promoters. Numerous constitutively active promoters which are operable in plants are available. A preferred example is the Cauliflower Mosaic Virus (CaMV) 35S promoter which is expressed constitutively in most plant tissues. In the alternative, the promoter may be a root-specific promoter or root cortex specific promoter, as explained in greater detail below.

313-36 (1987)).

[0060] As used herein, transformation refers to the introduction of exogenous DNA into cells; so as to produce transgenic cells stably transformed with the exogenous DNA.

[0061] Transformed cells are induced to regenerate intact tobacco plants through application of tobacco cell and tissue culture techniques that are well known in the art. The method of plant regeneration is chosen so as to be compatible with the method of transformation. The stable presence and the orientation of the QPRTase sequence in transgenic tobacco plants can be verified by Mendelian inheritance of the QPRTase sequence, as revealed by standard methods of DNA analysis applied to progeny resulting from controlled crosses. After regeneration of transgenic tobacco plants from transformed cells, the introduced DNA sequence is readily transferred to other tobacco varieties through conventional plant breeding practices and without undue experimentation.

[0062] For example, to analyze the segregation of the transgene, regenerated transformed plants (R_0) may be grown to maturity, tested for nicotine levels, and selfed to produce R_1 plants. A percentage of R_1 plants carrying the transgene are homozygous for the transgene. To identify homozygous R_1 plants, transgenic R_1 plants are grown to maturity and selfed. Homozygous R_1 plants will produce R_2 progeny where each progeny plant carries the transgene; progeny of heterozygous R_1 plants will segregate 3:1.

[0063] As nicotine serves as a natural pesticide which helps protect tobacco plants from damage by pests. It may therefor be desirable to additionally transform low or no nicotine plants produced by the present methods with a transgene (such as *Bacillus thuringiensis)* that will confer additional insect protection.

[0064] A preferred plant for use in the present methods are species of *Nicotiana*, or tobacco, including *N. tabacum, N. rustica and N. glutinosa*. Any strain or variety of tobacco may be used. Preferred are strains that are already low in nicotine content, such as *Nic1/Nic2* double mutants.

[0065] Any plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a vector of the present invention. The term "organogenesis," as used herein, means a process by which shoots and roots are developed sequentially from meristematic centers; the term "embryogenesis," as used herein, means a process by which shoots and roots develop together in a concerted fashion (not sequentially), whether from somatic cells or gametes. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, callus tissue, existing meristematic tissue (e.g., apical meristems, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem).

[0066] Plants of the present invention may take a variety of forms. The plants may be chimeras of transformed cells and non-transformed cells; the plants may be clonal transformants (e.g., all cells transformed to contain the transcription cassette); the plants may comprise grafts of transformed and untransformed tissues (e.g., a transformed root stock grafted to an untransformed scion in citrus species). The transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, first generation (or T1) transformed plants may be selfed to give homozygous second generation (or T2) transformed plants, and the T2 plants further propagated through classical breeding techniques. A dominant selectable marker (such as *npt*II) can be associated with the transcription cassette to assist in breeding.

[0067] In view of the foregoing, it will be apparent that plants which may be employed in practicing the present invention include those of the genus *Nicotiana*.

[0068] Those familiar with the recombinant DNA methods described above will recognize that one can employ a full-length QPRTase cDNA molecule or a full-length QPRTase chromosomal gene, joined in the sense orientation, with appropriate operably linked regulatory sequences, to construct transgenic tobacco cells and plants. (Those of skill in the art will also recognize that appropriate regulatory sequences for expression of genes in the sense orientation include any one of the known eukaryotic translation start sequences, in addition to the promoter and polyadenylation/transcription termination sequences described above). Such transformed tobacco plants are characterized by increased levels of QPRTase, and thus by higher nicotine content than untransformed control tobacco plants.

[0069] It should be understood, therefore, that use of QPRTase DNA sequences to decrease or to increase levels of QPRT enzyme, and thereby to decrease or increase the nicotine content in tobacco plants, falls within the scope of the present invention.

[0070] As used herein, a crop comprises a plurality of plants of the present invention, and of the same genus, planted together in an agricultural field. By "agricultural field" is meant a common plot of soil or a greenhouse. Thus, the present invention provides a method of producing a crop of plants having altered QPTRase activity and thus having increased or decreased nicotine levels, compared to a similar crop of non-transformed plants of the same species and variety.

[0071] The examples which follow are set forth to illustrate the present invention, and are not to be construed as limiting thereof.

nicotine level). There was a close correlation between nicotine levels and *TobRD2* transcript levels.

EXAMPLE 4

The Effect of Topping on *TobRD2* mRNA Levels

[0080] It is well known in the art that removal of the flower head of a tobacco plant (topping) increases root growth and increases nicotine content of the leaves of that plant. Topping of the plant and is a standard practice in commercial tobacco cultivation, and the optimal time for topping a given tobacco plant under a known set of growing conditions can readily be determined by one of ordinary skill in the art.

[0081] Tobacco plants (*N. tabacum* SR1) were grown from seed in soil for a month and transferred to pots containing sand. Plants were grown in a greenhouse for another two months until they started setting flowers. Flower heads and two nodes were then removed from four plants (topping). A portion of the roots was harvested from each plant after the indicated time and pooled for RNA extraction. Control plants were not decapitated. Total RNA (1μg) from each time point was electrophoresed through a 1% agarose gel containing 1.1M formaldehyde and transferred to a nylon membrane according to Sambrook, et al. (1989). The membranes were hybridized with ^{32}P-labeled TobRD2 cDNA fragments. Relative intensity of TobRD2 transcripts were measured by densitometry. Figure 6 illustrates the relative transcript levels (compared to zero time) for each time-point with topping (solid bars) or without topping (hatched bars).

[0082] Relative *TobRD2* levels were determined in root tissue over 24 hours; results are shown in **Figure 6** (solid bars indicate *TobRD2* transcript levels in topped plants; hatched bars indicate the *TobRD2* transcript levels in non-topped controls). Within six hours of topping of tobacco plants, mRNA levels of *TobRD2* increased approximately eight-fold in the topped plants; no increase was seen in control plants over the same time period.

EXAMPLE 5

Complementation of Bacterial Mutant

Lacking QPRTase with DNA of SEQ ID NO:1

[0083] *Escherichia coli* strain TH265 is a mutant lacking quinolate phosphoribosyl transferase (*nadC*-), and therefor cannot grow on media lacking nicotinic acids.

[0084] TH265 cells were transformed with an expression vector (pWS161) containing DNA of **SEQ ID NO:1**, or transformed with the expression vector (pKK233) only. Growth of the transformed bacteria was compared to growth of TH265 (pKK233) transformants, and to growth of the untransformed TH265 *nadC*- mutant. Growth was compared on ME minimal media (lacking nicotinic acid) and on ME minimal media with added nicotinic acid.

[0085] The *E. coli* strain with the QPTase mutation (*nadC*), TH265, was kindly provided by Dr. K.T. Hughes (Hughes et al., *J. Bact.* 175:479 (1993). The cells were maintained on LB media and competent cells prepared as described in Sambrook et al (1989). An expression plasmid was constructed in pKK2233 (Brosius, 1984) with the TobRD2 cDNA cloned under the control of the Tac promoter. The resulting plasmid, pWS161, was transformed into TH265 cells. The transformed cells were then plated on minimal media (Vogel and Bonner, 1956) agar plates with or without nicotinic acid (0.0002%) as supplement. TH265 cells alone and TH265 transformed with pKK2233 were plated on similar plates for use as controls.

[0086] Results are shown in Figure 4. Only the TH265 transformed with DNA of **SEQ ID NO:1** grew in media lacking nicotinic acid. These results show that expression of DNA of **SEQ ID NO:1** in TH265 bacterial cells conferred the *NadC*+ phenotype on these cells, confirming that this sequence encodes QPRTase. The TobRD2 nomenclature was thus changed to *NtQPT1*.

EXAMPLE 6

Transformation of Tobacco Plants

[0087] DNA of SEQ ID NO:1, in antisense orientation, is operably linked to a plant promoter (CaMV 35S or TobRD2 root-cortex specific promoter) to produce two different DNA cassettes: CaMV35S promoter/antisense SEQ ID NO:1 and TobRD2 promoter/antisense SEQ ID NO:1.

[0088] A wild-type tobacco line and a low-nicotine tobacco line are selected for transformation, e.g., wild-type Burley 21 tobacco (*Nic1+/Nic2+*) and homozygous *nic1-/nic2-* Burley 21. A plurality of tobacco plant cells from each line are transformed using each of the DNA cassettes. Transformation is conducted using an *Agrobacterium* vector, e.g., an *Agrobacterium-binary* vector carrying Ti-border sequences and the *nptII* gene (conferring resistance to kanamycin and

(A) LENGTH: 1399 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 52..1104

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GGA TTA CGT TTG GTG GAT AAA TGG GCG GTA TTG ATC GGT GGG GGG AAG                633
Gly Leu Arg Leu Val Asp Lys Trp Ala Val Leu Ile Gly Gly Gly Lys
    180                 185                 190

AAT CAC AGA ATG GGC TTA TTT GAT ATG GTA ATG ATA AAA GAC AAT CAC               681
Asn His Arg Met Gly Leu Phe Asp Met Val Met Ile Lys Asp Asn His
195                 200                 205                 210

ATA TCT GCT GCT GGA GGT GTC GGC AAA GCT CTA AAA TCT GTG GAT CAG               729
Ile Ser Ala Ala Gly Gly Val Gly Lys Ala Leu Lys Ser Val Asp Gln
                215                 220                 225

TAT TTG GAG CAA AAT AAA CTT CAA ATA GGG GTT GAG GTT GAA ACC AGG               777
Tyr Leu Glu Gln Asn Lys Leu Gln Ile Gly Val Glu Val Glu Thr Arg
            230                 235                 240

ACA ATT GAA GAA GTA CGT GAG GTT CTA GAC TAT GCA TCT CAA ACA AAG               825
Thr Ile Glu Glu Val Arg Glu Val Leu Asp Tyr Ala Ser Gln Thr Lys
        245                 250                 255

ACT TCG TTG ACT AGG ATA ATG CTG GAC AAT ATG GTT GTT CCA TTA TCT               873
Thr Ser Leu Thr Arg Ile Met Leu Asp Asn Met Val Val Pro Leu Ser
    260                 265                 270

AAC GGA GAT ATT GAT GTA TCC ATG CTT AAG GAG GCT GTA GAA TTG ATC               921
Asn Gly Asp Ile Asp Val Ser Met Leu Lys Glu Ala Val Glu Leu Ile
275                 280                 285                 290

AAT GGG AGG TTT GAT ACG GAG GCT TCA GGA AAT GTT ACC CTT GAA ACA               969
Asn Gly Arg Phe Asp Thr Glu Ala Ser Gly Asn Val Thr Leu Glu Thr
                295                 300                 305

GTA CAC AAG ATT GGA CAA ACT GGT GTT ACC TAC ATT TCT AGT GGT GCC              1017
Val His Lys Ile Gly Gln Thr Gly Val Thr Tyr Ile Ser Ser Gly Ala
            310                 315                 320

CTG ACG CAT TCC GTG AAA GCA CTT GAC ATT TCC CTG AAG ATC GAT ACA              1065
Leu Thr His Ser Val Lys Ala Leu Asp Ile Ser Leu Lys Ile Asp Thr
        325                 330                 335

GAG CTC GCC CTT GAA GTT GGA AGG CGT ACA AAA CGA GCA TGAGCGCCAT               1114
Glu Leu Ala Leu Glu Val Gly Arg Arg Thr Lys Arg Ala
    340                 345                 350

TACTTCTGCT ATAGGGTTGG AGTAAAAGCA GCTGAATAGC TGAAAGGTGC AAATAAGAAT           1174

CATTTTACTA GTTGTCAAAC AAAAGATCCT TCACTGTGTA ATCAAACAAA AAGATGTAAA           1234

TTGCTGGAAT ATCTCAGATG GCTCTTTTCC AACCTTATTG CTTGAGTTGG TAATTTCATT           1294

ATAGCTTTGT TTTCATGTTT CATGGAATTT GTTACAATGA AAATACTTGA TTTATAAGTT           1354

TGGTGTATGT AAAATTCTGT GTTACTTCAA ATATTTTGAG ATGTT                            1399
```

(2) INFORMATION FOR SEQ ID NO:2:

Thr Arg Thr Ile Glu Glu Val Arg Glu Val Leu Asp Tyr Ala Ser Gln
245 250 255

Thr Lys Thr Ser Leu Thr Arg Ile Met Leu Asp Asn Met Val Val Pro
260 265 270

Leu Ser Asn Gly Asp Ile Asp Val Ser Met Leu Lys Glu Ala Val Glu
275 280 285

Leu Ile Asn Gly Arg Phe Asp Thr Glu Ala Ser Gly Asn Val Thr Leu
290 295 300

Glu Thr Val His Lys Ile Gly Gln Thr Gly Val Thr Tyr Ile Ser Ser
305 310 315 320

Gly Ala Leu Thr His Ser Val Lys Ala Leu Asp Ile Ser Leu Lys Ile
325 330 335

Asp Thr Glu Leu Ala Leu Glu Val Gly Arg Arg Thr Lys Arg Ala
340 345 350

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1053 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGTTTAGAG CTATTCCTTT CACTGCTACA GTGCATCCTT ATGCAATTAC AGCTCCAAGG 60

TTGGTGGTGA AAATGTCAGC AATAGCCACC AAGAATACAA GAGTGGAGTC ATTAGAGGTG 120

AAACCACCAG CACACCCAAC TTATGATTTA AAGGAAGTTA TGAAACTTGC ACTCTCTGAA 180

GATGCTGGGA ATTTAGGAGA TGTGACTTGT AAGGCGACAA TTCCTCTTGA TATGGAATCC 240

GATGCTCATT TTCTAGCAAA GGAAGACGGG ATCATAGCAG GAATTGCACT TGCTGAGATG 300

ATATTCGCGG AAGTTGATCC TTCATTAAAG GTGGAGTGGT ATGTAAATGA TGGCGATAAA 360

GTTCATAAAG GCTTGAAATT TGGCAAAGTA CAAGGAAACG CTTACAACAT TGTTATAGCT 420

GAGAGGGTTG TTCTCAATTT TATGCAAAGA ATGAGTGGAA TAGCTACACT AACTAAGGAA 480

ATGGCAGATG CTGCACACCC TGCTTACATC TTGGAGACTA GGAAAACTGC TCCTGGATTA 540

CGTTTGGTGG ATAAATGGGC GGTATTGATC GGTGGGGGGA AGAATCACAG AATGGGCTTA 600

11. A plant cell containing a DNA construct according to any of claims 2 to 10.

12. A transgenic plant comprising plant cells according to claim 11.

13. A peptide having **SEQ ID NO:2.**

14. A peptide encoded by a DNA sequence selected from the group consisting of:

(a) SEQ ID NO:1;
(b) DNA sequences which have at least 65% homology to isolated DNA of (a) above and which encode a quinolate phosphoribosyl transferase enzyme; and
(c) DNA sequences which differ from the DNA of (a) or (b) above due to the degeneracy of the genetic code.

15. A method of making a transgenic plant cell having reduced quinolate phosphoribosyl transferase (QPRTase) expression, said method comprising:

providing a plant cell of a type known to express quinolate phosphoribosyl transferase;
providing an exogenous DNA construct, which construct comprises, in the 5' to 3' direction, a promoter operable in a plant cell and a DNA sequence encoding quinolate phosphoribosyl transferase mRNA and selected from the DNA sequences of claim 1, said DNA operably associated with said promoter; and
transforming said plant cell with said DNA construct to produce transformed cells, said plant cell having reduced expression of QPRTase compared to an untransformed cell.

16. The method of claim 15, wherein said DNA comprising a portion of sequence encoding quinolate phosphoribosyl transferase mRNA selected from the DNA sequences of claim 1 is in sense or antisene orientation.

17. The method of claim 15 or claim 16 wherein said plant cell is *Nicotiana tabacum.*

18. The method of any of claims 15 to 17, further comprising regenerating a plant from said transformed plant cell.

19. A method according to any of claims 15 to 18, wherein said promoter is constitutively active.

20. A method according to any of claims 15 to 18, wherein said promoter is selectively active in plant root tissue cells or in plant root cortex tissue cells.

21. A method according to any of claims 15 to 20, wherein said transforming step is carried out by bombarding said plant cell with microparticles carrying said DNA construct.

22. A method according to any of claims 15 to 20 wherein said transforming step is carried out by infecting said plant cell with an *Agrobacterium tumefaciens* containing a Ti plasmid carrying said DNA construct.

23. A method of producing transgenic tobacco seeds, comprising collecting seed from a transgenic tobacco plant produced by the method of claim 17.

24. The method according to any of claims 15 to 23, wherein said exogenous DNA sequence comprises a quinolate phosphoribosyl transferase encoding sequence and is selected from the DNA sequences of claim 1 and is complementary to said quinolate phosphoribosyl transferase messenger RNA (QPRT mRNA) expressed in said plant cell in a region selected from:

(a) the 5'-untranslated sequence of said QPRT mRNA;
(b) the 3 '-untranslated sequence of said QPRT mRNA; and
(c) the translated region of said QPRT mRNA.

25. The method according to any of claims 15 to 23, wherein said exogenous DNA sequence comprises a quinolate phosphoribosyl transferase encoding sequence and is selected from the DNA sequences of claim 1 and is complementary to at least 15 nucleotides of said quinolate phosphoribosyl transferase messenger RNA expressed in said plant cell.

36. A transgenic plant of the species *Nicotiana* having increased quinolate phosphoribosyl transferase (QPRTase) expression relative to a non-transformed control plant, said transgenic plant comprising transgenic plant cells containing:

an exogenous DNA construct comprising, in the 5' to 3' direction, a promoter operable in said plant cell and a DNA sequence encoding a plant quinolate phosphoribosyl transferase selected from the DNA sequences of claim 1, said DNA operably associated with said promoter;
said plant exhibiting increased QPRTase expression compared to a non-transformed control plant.

37. A transgenic plant of the species *Nicotiana* having increased quinolate phosphoribosyl transferase (QPRTase) expression relative to a non-transformed control plant, wherein said transgenic plant is a progeny of a plant according to claim 36 and comprises the transgene according to claim 1.

38. A method for increasing expression of a quinolate phosphoribosyl transferase gene in a plant cell, said method comprising:

growing a plant cell transformed to contain exogenous DNA selected from the DNA sequences of claim 1 wherein said exogenous DNA encodes quinolate phosphoribosyl transferase.

39. The method according to claim 38, wherein said transformed plant cell is obtained by a method comprising:

integrating into the genome of a host plant cell a construct comprising, in a direction of transcription, a promoter functional in said plant cell, a DNA sequence encoding quinolate phosphoribosyl transferase functional in said cell selected from the DNA sequences of claim 1, said DNA sequence operably associated with said promoter, and a transcriptional termination region functional in said cell, whereby a transformed plant cell is obtained.

40. A method of producing a tobacco plant having increased levels of nicotine in leaves of said tobacco plant, said method comprising:

growing a tobacco plant, or progeny plants thereof, wherein said plant comprises cells containing a DNA construct comprising a transcriptional initiation region functional in said plant and an exogenous DNA sequence selected from the DNA sequences of claim 1 operably joined to said transcriptional initiation region,

wherein said DNA sequence encodes quinolate phosphoribosyl transferase functional in said cells.

Patentansprüche

1. Isoliertes DNA Molekül, welches eine Sequenz aufweist, die aus der Gruppe ausgewählt ist, welche aus folgendem besteht:

(a) SEQ ID NO:1;
(b) DNA Sequenzen, welche ein Enzym mit SEQ ID NO: 2 kodieren;
(c) DNA Sequenzen, die wenigstens 65% Homologie mit einer isolierten DNA aus obigem (a) oder (b) aufweisen und welche ein Chinolatphosphoribosyltransferase Enzym kodieren; und
(d) DNA Sequenzen, die sich von der DNA aus obigem (a), (b) oder (c) anhand der Degenerierung des genetischen Kodes unterscheiden.

2. DNA Konstrukt mit einer Expressionskassette, welches Konstrukt in der 5' - 3' Richtung einen in pflanzlichen Zellen funktionellen Promotor und eine DNA Sequenz mit einer eine Chinolatphosphoribosyltransferase kodierenden Sequenz nach Anspruch 1 beinhaltet, welche abwärts von dem genannten Promotor positioniert und funktionell mit diesem verbunden ist.

3. DNA Konstrukt mit einer Expressionskassette, welches Konstrukt in der 5' - 3' Richtung einen pflanzlichen Promotor und eine DNA Sequenz mit einer eine Chinolatphosphoribosyltransferase kodierenden Sequenz nach Anspruch 1 beinhaltet, welche abwärts von dem genannten Promotor positioniert und funktionell mit diesem verbunden ist, wobei die genannte DNA Sequenz in antisense Orientierung ist.

19. Verfahren nach einem der Ansprüche 15 bis 18, wobei der genannte Promotor konstitutiv aktiv ist.

20. Verfahren nach einem der Ansprüche 15 bis 18, wobei der genannte Promotor selektiv in Zellen des pflanzlichen Wurzelgewebes oder des pflanzlichen Wurzelcortexgewebes aktiv ist.

21. Verfahren nach einem der Ansprüche 15 bis 20, wobei der genannte Tranfsormationsschritt durch Bombardieren der genannten pflanzlichen Zelle mit Mikropartikelchen, welche genannte DNA tragen, durchgeführt wird.

22. Verfahren nach einem der Ansprüche 15 bis 20, wobei der genannte Tranfsormationsschritt durch Infizierung der genannten pflanzlichen Zelle mit einem *Agrobacterium tumefaciens* durchgeführt wird, welcher ein das genannte DNA Konstrukt tragendes Ti Plasmid enthält.

23. Verfahren zur Produktion von transgenen Tabaksamen, welche das Ernten von Samen transgener Tabakpflanzen umfaßt, die nach dem Verfahren nach Anspruch 17 erzeugt wurden.

24. Verfahren nach einem der Ansprüche 15 bis 23, wobei die genannte exogene DNA Sequenz eine Chinolatphosphoribosyltransferase Enzym kodierende Sequenz aufweist, aus den DNA Sequenzen des Anspruchs 1 ausgewählt ist und komplementär ist mit der genannten Chinolatphosphoribosyltransferase - Messenger - RNA (CPRT mRNA), welche in der genannten Pflanzenzelle in einer Region exprimiert ist, die ausgewählt ist aus:

(a) der 5'- nicht translatierten Sequenz der genannten CPRT mRNA;
(b) der 3'- nicht translatierten Sequenz der genannten CPRT mRNA; und
(c) der translatierten Region der genannten CPRT mRNA.

25. Verfahren nach einem der Ansprüche 15 bis 23, wobei die genannte exogene DNA Sequenz eine Chinolatphosphoribosyltransferase Enzym kodierende Sequenz aufweist, aus den DNA Sequenzen des Anspruchs 1 ausgewählt ist und komplementär ist mit mindestens 15 Nukleotiden der genannten Chinolatphosphoribosyltransferase - Messenger - RNA, die in der genannten pflanzlichen Zelle exprimiert ist.

26. Verfahren nach einem der Ansprüche 15 bis 23, wobei die genannte exogene DNA Sequenz eine Chinolatphosphoribosyltransferase Enzym kodierende Sequenz aufweist, aus den DNA Sequenzen des Anspruchs 1 ausgewählt ist und komplementär ist mit mindestens 200 Nukleotiden der genannten Chinolatphosphoribosyltransferase - Messenger - RNA, die in der genannten pflanzlichen Zelle exprimiert ist.

27. Verfahren nach einem der Ansprüche 15 bis 23, wobei genannte exogene DNA Sequenz eine Chinolatphosphoribosyltransferase kodierende Sequenz beinhaltet, welche von den DNA Sequenzen nach Anspruch 1 ausgewählt ist.

28. Transgene Pflanze der Art *Nicotiana* mit reduzierter Chinolatphosphoribosyltransferase Expression in Bezug auf eine nicht transformierte Kontrollpflanze, welche genannte transgene Pflanze pflanzliche Zellen aufweist, welche folgendes enthalten:

ein exogenes DNA Konstrukt, umfassend in 5' - 3' Richtung einen in pflanzlichen Zellen funktionellen Promotor und DNA, welche ein Segment einer DNA Sequenz beinhaltet, die pflanzliche Chinolatphosphoribosyltransferase mRNA' kodiert und aus den DNA Sequenzen den Anspruchs 1 ausgewählt ist, wobei die genannte DNA funktionell mit dem genannten Promotor verbunden ist;

wobei die genannte Pflanze eine reduzierte Chinolatphosphoribosyltransferase Expression im Vergleich zu einer nicht transformierten Kontrollpflanze aufweist.

29. Pflanze nach Anspruch 28, wobei das genannte DNA Segment, welches ein Segment von Chinolatphosphoribosyltransferase mRNA kodierender DNA Sequenz beinhaltet, aus den DNA Sequenzen den Anspruchs 1 ausgewählt ist und in Leserichtung oder antisense Orientierung ist.

30. Transgene Pflanze der Art *Nicotiana*, welche eine reduzierte Chinolatphosphoribosyltransferase (CPRTase) Expression in Bezug auf eine nicht transformierte Kontrollpflanze aufweist, wobei die genannte transgene Pflanze ein Nachkomme einer Pflanze nach Anspruch 28 ist und das Transgen nach Anspruch 1 aufweist.

Kultivierung einer pflanzlichen Zelle, welche transformiert ist, um eine aus den DNA Sequenzen des Anspruchs 1 ausgewählte, exogene DNA zu enthalten, wobei die genannte exogene DNA Chinolatphosphoribosyltransferase kodiert.

39. Verfahren nach Anspruch 38, wobei die genannte transformierte pflanzliche Zelle durch ein Verfahren erhalten wird, welches folgendes umfaßt:

Integrieren in das Genom einer pflanzlichen Wirtszelle eines Konstrukts, welches in der Transkriptionsrichtung einen in der genannten pflanzlichen Zelle funktionsfähigen Promotor, eine in der genannten Zelle funktionsfähige Chinolatphosphoribosyltransferase kodierende DNA Sequenz, welche aus den DNA Sequenzen des Anspruchs 1 ausgewählt und funktionell mit genanntem Promotor verbunden ist, und eine in genannter Zelle funktionsfähige transkriptionsterminierende Region umfaßt, wodurch eine transformierte pflanzliche Zelle erhalten wird.

40. Verfahren zur Herstellung von Tabakpflanze, welche erhöhte Werte von Nikotin in Blättern der genannten Tabakpflanze aufweisen, welches folgendes umfaßt:

Kultivierung einer Tabakpflanze oder deren Nachkommen, wobei die genannten pflanzlichen Zellen ein DNA Konstrukt umfassen, welches eine in der genannten Zelle funktionsfähige transkriptionsinitiierende Region und eine exogene DNA Sequenz aufweist, die funktionell mit der genannten transkriptionsinitiierender Region verbunden und aus den DNA Sequenzen des Anspruchs 1 ausgewählt ist, wobei die genannte DNA Sequenz eine in den genannten Zellen funktionsfähige Chinolatphosphoribosyltransferase kodiert.

Revendications

1. Molécule isolée d'ADN comprenant une séquence choisie parmi le groupe consistant en :

(a) SEQ ID N°1 ;
(b) Séquences d'ADN qui encodent un enzyme ayant SEQ ID N°2 ;
(c) Séquences d'ADN qui ont au moins 65% d'homologie avec l'ADN isolé de (a) ou (b) ci-dessus et qui encodent un enzyme transférase phosphoribosyle quinolate ; et
(d) Séquences d'ADN qui diffèrent de l'ADN de (a), (b) ou (c) ci-dessus, du fait de la dégénérescence du code génétique.

2. Motif d'ADN comprenant une cassette d'expression, comportant dans la direction 5' à 3' un promoteur activable dans une cellule de plante et une séquence ADN comprenant une séquence transférase phosphoribosyle quinolate d'encodage selon la revendication 1, positionné en aval dudit promoteur et associé de manière opérationnelle à celui-ci.

3. Motif d'ADN comprenant une cassette d'expression, comportant dans la direction 5' à 3' un promoteur de plante et une séquence ADN comprenant une séquence transférase phosphoribosyle quinolate d'encodage selon la revendication 1, disposé en aval dudit promoteur et lié de manière opérationnelle à celui-ci, ladite séquence ADN étant dans une orientation de sens inverse.

4. Motif d'ADN comprenant, dans la direction 5' à 3', un promoteur activable dans une cellule de plante et encodant ADN une transférase phosphoribosyle quinolate de plante, choisie parmi les séquences ADN selon la revendication 1, ledit ADN étant associé de manière opérationnelle audit promoteur.

5. Motif d'ADN comprenant, dans la direction 5' à 3', un promoteur activable dans une cellule de plante et encodant ADN une transférase phosphoribosyle quinolate de plante, choisie parmi les séquences ADN selon la revendication 1, ledit ADN étant dans une orientation en sens inverse et associé de manière opérationnelle audit promoteur.

6. Motif d'ADN selon la revendication 2, 3, 4 ou 5, ledit promoteur étant actif de manière constitutive dans des cellules de plante.

7. Motif d'ADN selon la revendication 2, 3, 4 ou 5, **caractérisé en ce que** ledit promoteur est actif de manière sélective dans des cellules de tissus de racine de plante ou dans des cellules de tissus de cortex de racines de plante.

(a) la séquence 5' non translatée dudit QPRT mRNA ;
(b) la séquence 3' non translatée dudit QPRT mRNA ; et
(c) la région translatée dudit QPRT MRNA.

25. Procédé selon l'une des revendications 15 à 23, **caractérisé en ce que** ladite séquence exogène ADN, comporte une séquence d'encodage transférase phosphoribosyle quinolate et est choisie parmi les séquences ADN de la revendication 1, et est complémentaire à au moins 15 nucléotides dudit messager RNA transférase phosphoribosyle quinolate exprimé dans ladite cellule de plante.

26. Procédé selon l'une des revendications 15 à 23, **caractérisé en ce que** ladite séquence exogène ADN, comporte une séquence d'encodage transférase phosphoribosyle quinolate et est choisie parmi les séquences ADN de la revendication 1, et est complémentaire à au moins 200 nucléotides dudit messager RNA transférase phosphoribosyle quinolate exprimé dans ladite cellule de plante.

27. Procédé selon l'une des revendications 15 à 23, **caractérisé en ce que** ladite séquence ADN exogène comporte une transférase phosphoribosyle quinolate encodant une séquence choisie à partir des séquences ADN de la revendication 1.

28. Plante transgénique de l'espèce *Nicotiana* ayant une transférase phosphoribosyle quinolate réduite (QPRTase) d'expression, relative à une plante de contrôle non transformée, ladite plante transgénique comprenant des cellules de plante transgéniques contenant :

- un motif ADN exogène comprenant, dans la direction 5' à 3', un promoteur susceptible d'opérer dans ladite cellule de plante et un ADN comprenant un segment d'une séquence ADN qui encode une transférase phosphoribosyle quinolate mRNA de plante, choisie parmi les séquences ADN de la revendication 1, ledit ADN étant associé de manière opérationnelle audit promoteur ;
- ladite plante montrant une expression réduite QPRTase comparée à une plante de contrôle non transformée.

29. Procédé selon la revendication 28, **caractérisé en ce que** ledit segment d'ADN, comportant un segment d'une séquence ADN encodant une transférase phosphoribosyle quinolate mRNA, est choisie parmi les séquences ADN de la revendication 1, et est dans une orientation en sens inverse ou dans une orientation de même sens.

30. Plante transgénique de l'espèce *Nicotiana* ayant une expression réduite (QPRTase) transférase phosphoribosyle quinolate, par rapport à une plante de contrôle non transformée, **caractérisée en ce que** ladite plante transgénique est un progène d'une plante selon la revendication 28 et comprend le transgène selon la revendication 1.

31. Semence de plante transgénique de l'espèce *Nicotiana* ayant une expression réduite (QPRTase) transférase phosphoribosyle quinolate, par rapport à une plante de contrôle non transformée, **caractérisée en ce que** ladite plante transgénique est une plante selon la revendication 28 ou un progène de celle-ci et dont les semences comprennent le transgène selon la revendication 1.

32. Culture comprenant une pluralité de plantes selon la revendication 28 plantées ensemble dans un terrain agricole.

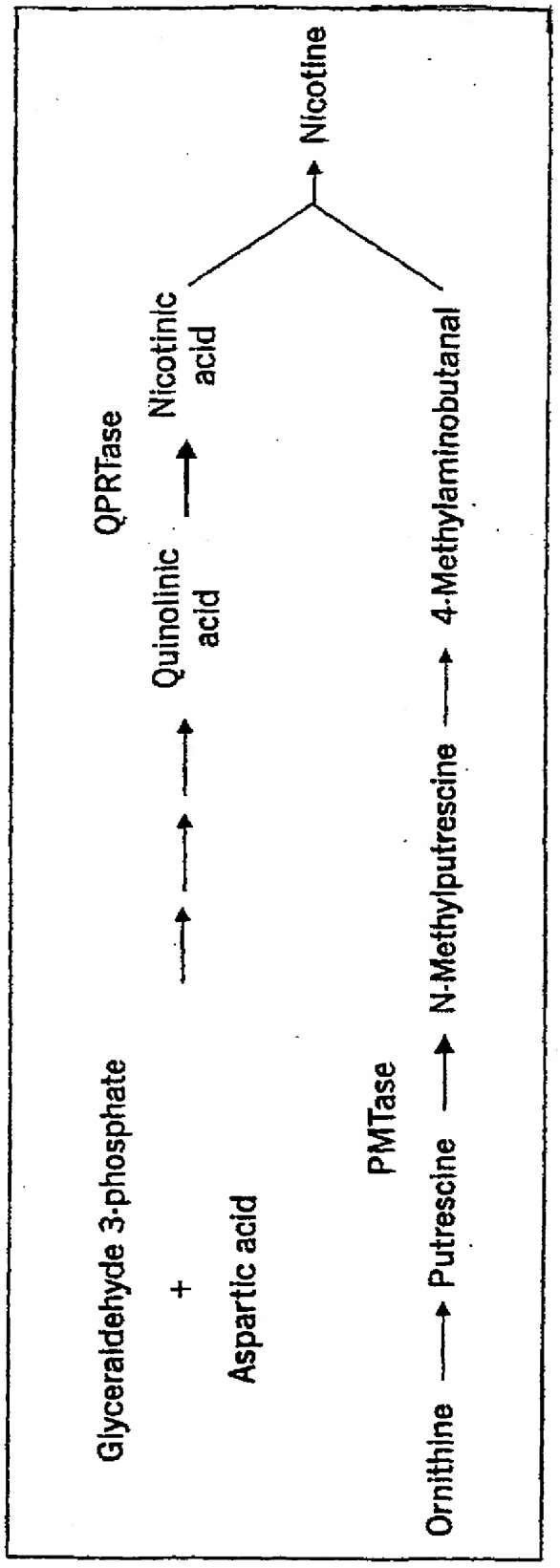
33. Procédé pour réduire l'expression d'un gène transférase phosphoribosyle quinolate dans une cellule de plante, ledit procédé comprenant :

- faire pousser une cellule de plante transformée pour contenir un ADN exogène choisi parmi les séquences ADN de la revendication 1, une chaîne transcrite dudit ADN exogène étant complémentaire de transférase phosphoribosyle quinolate mRNA, endogène à ladite cellule, tandis que la transcription de ladite chaîne complémentaire réduit l'expression dudit gène transférase phosphoribosyle quinolate.

34. Procédé pour produire une plante de tabac ayant des niveaux diminués de nicotine dans les feuilles de ladite plante de tabac, ledit procédé comprend :

- faire pousser une plante de tabac, ou des plants de progène de celle-ci, ladite plante comportant des cellules contenant un motif ADN incorporant une région d'initiation de transcription, fonctionnel dans ladite plante et une séquence ADN dans ladite plante et une séquence ADN exogène, choisi parmi les séquences ADN de la revendication 1, jointe de manière opérationnelle à ladite région d'initiation transcriptionnelle, tandis qu'une

FIG. 1


Glyceraldehyde 3-phosphate
+
Aspartic acid
Quinolinic acid
QPRTase
Nicotinic acid
Nicotine
Ornithine
Putrescine
PMTase
N-Methylputrescine
4-Methylaminobutanal

```
caaaaactat tttccacaaa attcatttca caacccccc aaaaaaaaac cATGTTTAGA   60
GCTATTCCTT TCACTGCTAC AGTGCATCCT TATGCAATTA CAGCTCCAAG GTTGGTGGTG  120
AAAATGTCAG CAATAGCCAC CAAGAATACA AGAGTGGAGT CATTAGAGGT GAAACCACCA  180
GCACACCCAA CTTATGATTT AAAGGAAGTT ATGAAACTTG CACTCTCTGA AGATGCTGGG  240
TTTCTAGCAA AGGAAGACGG GATCATAGCA GGAATTGCAC TTGCTGAGAT GATATTCGCG  360
GAAGTTGATC CTTCATTAAA GGTGGAGTGG TATGTAAATG ATGGCGATAA AGTTCATAAA  420
GGCTTGAAAT TTGGCAAAGT ACAAGGAAAC GCTTACAACA TTGTTATAGC TGAGAGGGTT  480
GTTCTCAATT TTATGCAAAG AATGAGTGGA ATAGCTACAC TAACTAAGGA AATGGCAGAT  540
GCTGCACACC CTGCTTACAT CTTGGAGACT AGGAAAACTG CTCCTGGATT ACGTTTGGTG  600
GATAAATGGG CGGTATTGAT CGGTGGGGGG AAGAATCACA GAATGGGCTT ATTTGATATG  660
GTAATGATAA AAGACAATCA CATATCTGCT GCTGGAGGTG TCGGCAAAGC TCTAAAATCT  720
GTGGATCAGT ATTTGGAGCA AAATAAACTT CAAATAGGGG TTGAGGTTGA AACCAGGACA  780
ATTGAAGAAG TACGTGAGGT TCTAGACTAT GCATCTCAAA CAAAGACTTC GTTGACTAGG  840
ATAATGCTGG ACAATATGGT TGTTCCATTA TCTAACGGAG ATATTGATGT ATCCATGCTT  900
AAGGAGGCTG TAGAATTGAT CAATGGGAGG TTTGATACGG AGGCTTCAGG AAATGTTACC  960
CTTGAAACAG TACACAAGAT TGGACAAACT GGTGTTACCT ACATTTCTAG TGGTGCCCTG 1020
ACGCATTCCG TGAAAGCACT TGACATTTCC CTGAAGATCG ATACAGAGCT CGCCCTTGAA 1080
GTTGGAAGGC GTACAAAACG AGCATGAgcg ccattacttc tgctataggg ttggagtaaa 1140
agcagctgaa tagctgaaag gtgcaaataa gaatcatttt actagttgtc aaacaaaaga 1200
tccttcactg tgtaatcaaa caaaaagatg taaattgctg gaatatctca gatggctctt 1260
ttccaacctt attgcttgag ttggtaattt cattatagct ttgttttcat gtttcatgga 1320
atttgttaca atgaaaatac ttgatttata agtttggtgt atgtaaaatt ctgtgttact 1380
tcaaatattt tgagatgtt                                              1399
```

FIGURE 2A

```
MFRAIPFTAT VHPYAITAPR LVVKMSAIAT KNTRVESLEV KPPAHPTYDL   50
KEVMKLALSE DAGNLGDVTC KATIPLDMES DAHFLAKEDG IIAGIALAEM  100
IFAEVDPSLK VEWYVNDGDK VHKGLKFGKV QGNAYNIVIA ERVVLNFMQR  150
MSGIATLTKE MADAAHPAYI LETRKTAPGL RLVDKWAVLI GGGKNHRMGL  200
FDMVMIKDNH ISAAGGVGKA LKSVDQYLEQ NKLQIGVEVE TRTIEEVREV  250
LDYASQTKTS LTRIMLDNMV VPLSNGDIDV SMLKEAVELI NGRFDTEASG  300
NVTLETVHKI GQTGVTYISS GALTHSVKAL DISLKIDTEL ALEVGRRTKR  350
A                                                       351
```

FIGURE 2B

```
N. tabacum          MFRAIPFTATVHPYAITAPRLVVKMSAIATKNTRVESLEVKPPAHPTYDL
R. rubrum           *--------RPNH----------------------PVAALS*F----AI
M. leprae           *--------LSDC----------------------EFDAAR--------
S. typhimurium      *--------PPRR*NPDDR*----------DALL*RINLDI*A----AV
E. coli             *--------PPRR*NPDTR*----------DELL*RINLDI*G----AV
H. sapien           *---------------D*EG*ALLLPPVTLAALVDSWLREDC*G------
S. cerevisiae       *---------------PVYE-HLLPVNGAWRQDVTNWLSEDV*S------

N. tabacum          KEVMKLALSEDAGNLGDVTCKATIPLDMESDAHFLAKEDGIIAGIA----
R. rubrum           D*AVRR**A**L*RA**I*ST****AATRAH*RFV*RQP**L**LGCA--
M. leprae           -DTIRR**H**LRYGL*I*TQ**V*AGTVVTGSMVPR*P*VIAGVDVALL
S. typhimurium      AQALREDLGGEVDAGN*I*AQL-L*A*TQAH*TVITR*D*VF----CGKR
E. coli             AQALREDLGGTVDANN*I*A*L-L*ENSR*H*TVITR*N*VF----CGKR
H. sapien           ----------------LNYAALVSGAGP*QAALWAKSP*VL----AGQP
S. cerevisiae       ----------------FDFGGYVVGSDLKEANLYCKQD*ML----CGVP

N. tabacum          -LAEMIFAEVDPSLKVEWYVNDGDKVHKGLK------FGKVQGNAYNIVI
R. rubrum           --RSAF-ALLDDTVTFTTPLE**AEIAA*QT------VAE*A*A*RT*LA
M. leprae           VLD*VF-GVDGYRVLY--R*E**ARLQS*QP------LLTVQAA*RGLLT
S. typhimurium      WVE*VFIQLAGDDVRLT*H*D***AI*ANQT------VFELN*PARVLLT
E. coli             WVE*VFIQLAGDDVTII*H*D***VINANQS------LFELE*PSRVLLT
H. sapien           FFDAIFTQL---NCQVS*FLPE*S*LVPVAR------VAEVR*P*HDLLL
S. cerevisiae       FAW*VFNQC---ELQVE*LFKE*SFLEPSKNDSGKIVVAKIT*P*K**LL

N. tabacum          AERVVLNFMQRMSGIATLTKEMAD--AAH--PAYILETRKTAPGLRLVDK
R. rubrum           ***TA***LGHL*****R*RRFG*AI*HT--R*RLTC****T****GLE*
M. leprae           ***TM***VCHM*****V*VAWV*AVRGT--K*KIRD****L****ALQ*
S. typhimurium      G**TA***V*TL**VASEVRRYVGLL*GT--QTQL*D****L****TAL*
E. coli             G*PTA***V*TL**VASKVRHYVELLEGT--NTQL*D****L****SAL*
H. sapien           G***A**TLARC****SAAAAAVEAARGAGWTGHVAG****T**F***E*
S. cerevisiae       ***TA**ILSRS****TASHKIISLARSTGYKGTIAG****T****RLE*

N. tabacum          WAVLIGGGKNHRMGLFDMVMIKDNHISAAGGVGKALKSVDQYLEQNKLQI
R. rubrum           Y**RC***S***F**D*A*L******AVA***SA**SRAR-AGVGHMVRI
M. leprae           Y**RV***V***L**G*TAL*****VA*V*S*VD**RA*R-AAAPEL-PC
S. typhimurium      Y***C***A***L**T*AFL******I*S*S*RQ*VEKAF-W*HPD-APV
E. coli             Y***C***A***L**S*AFL******I*S*S*RQ*VEKAS-W*HPD-APV
H. sapien           YGL*V**AAS**YD*GGLVML*D**VVPP***EK*VRAARQ---AADFAL
S. cerevisiae       YSM*V**CDT**YD*SS**ML*D***W*T*SITN*V*NARA---VCGFAV
```

FIGURE 3

```
N. tabacum          GVEVETRTIEEVREVLDYASQTKTSLTRIMLDNMVVPLSNGDIDVSMLKE
R. rubrum           EI****--L*QLA***AVGGAEV-----VL****-----DAPT----*TR
M. leprae           E****S--L*QLDAM*A-EEPEL-----*L***F--*VWQTQV----AVQ
S. typhimurium      E****N--LDELDDA*K-*GADI-----*****F-----NTDQ----MR*
E. coli             E****N--L**LD*A*K-*GADI-----*****F-----ETEQ----MR*
H. sapien           K****CSSLQ**VQAAE-*GADL-----VL***F------KPEELHPTAT
S. cerevisiae       KI***CLSED*AT*AIE-*GADV-----*****F------KGDGLK*CAQ

N. tabacum          AVELI---NGRFDTEASGNVTLETVHKIG-QTGVTYISSGALTHSVKALD
R. rubrum           **DMV---A**LV*****G*S*D*IAALA-ES**D***V*******TT**
M. leprae           RRDIR---APTVLL*S**GLS**NAAIYA-G***DYLAV*******RI**
S. typhimurium      **KRV---**QARL*V*****AE*LREFA-E***DF**VG******R***
E. coli             **KRT---**KALL*V*****DK*LREFA-E***DF**V*******Q***
H. sapien           *LKAQFPSVA---VEA**GIT*DNLPQF-CGPHIDV**M*M**QA*P***
S. cerevisiae       SLKNKWNGKKHFLLEC**GLN*DNLEEYLCD-DIDIY*TSSIHQGTPVI*
```

		% Identity	% Similarity
N. tabacum	ISKLIDTELALEVGRRTKRA		
R. rubrum	*G*D*VVA-----PPKAERA	15.9	43.2
M. leprae	*G*DL	18.3	37.3
S. typhimurium	LSMRFC	18.2	34.8
E. coli	LSMRFR	17.9	32.8
H. sapien	F***L---F*K*VAPVP*IH	16.8	31.7
S. cerevisiae	F***LAH	14.6	27.8

FIGURE 3 continued

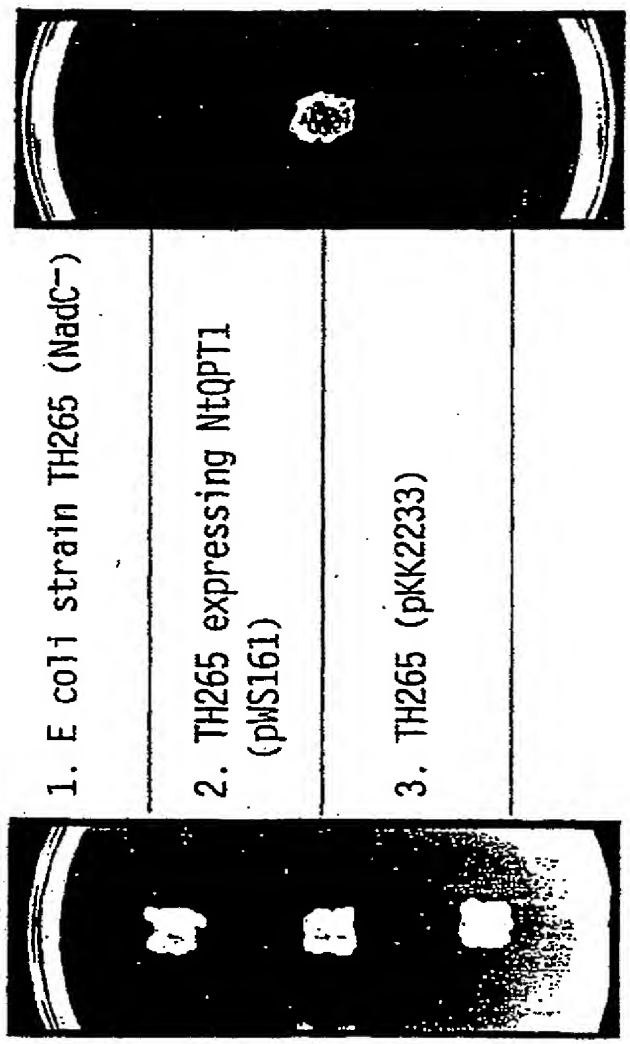

1. E coli strain TH265 (NadC-)
2. TH265 expressing NtQPT1 (pWS161)
3. TH265 (pKK2233)
ME Minimal Media
ME Minimal Media + Nicotinic Acid

FIG.4

FIG. 5

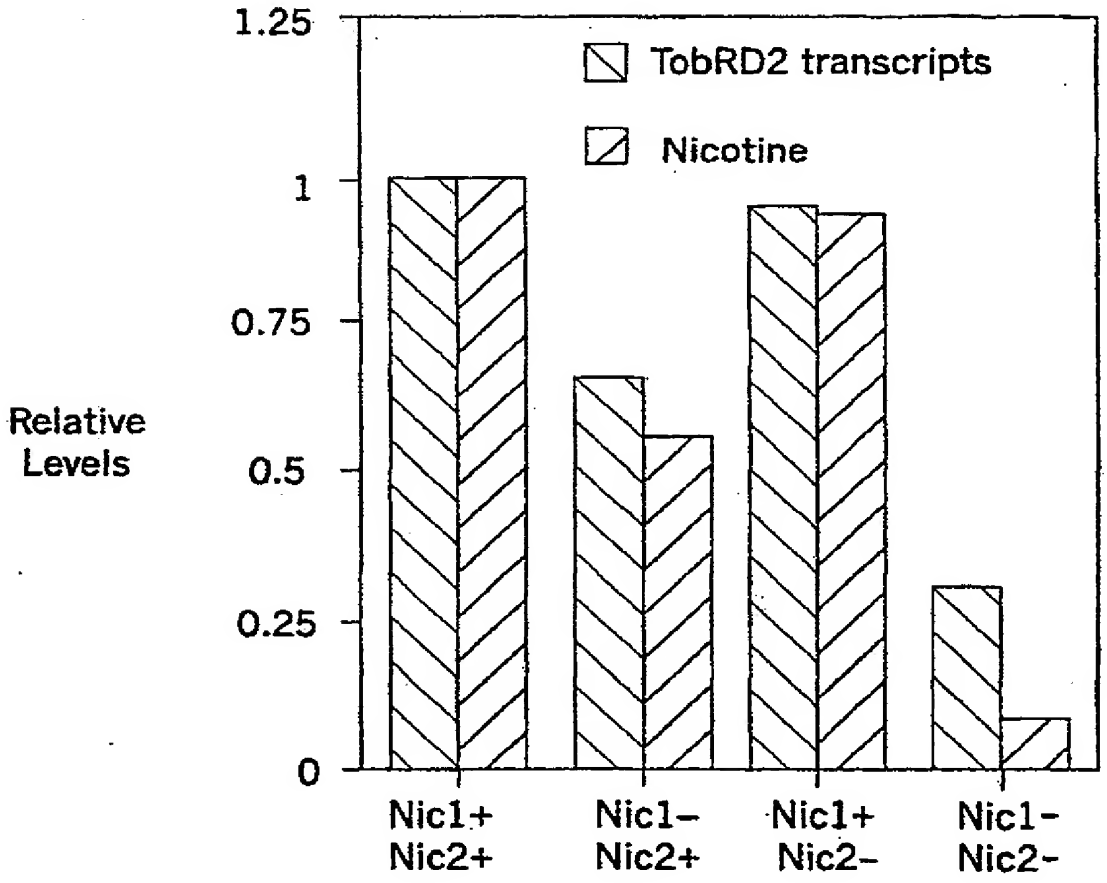
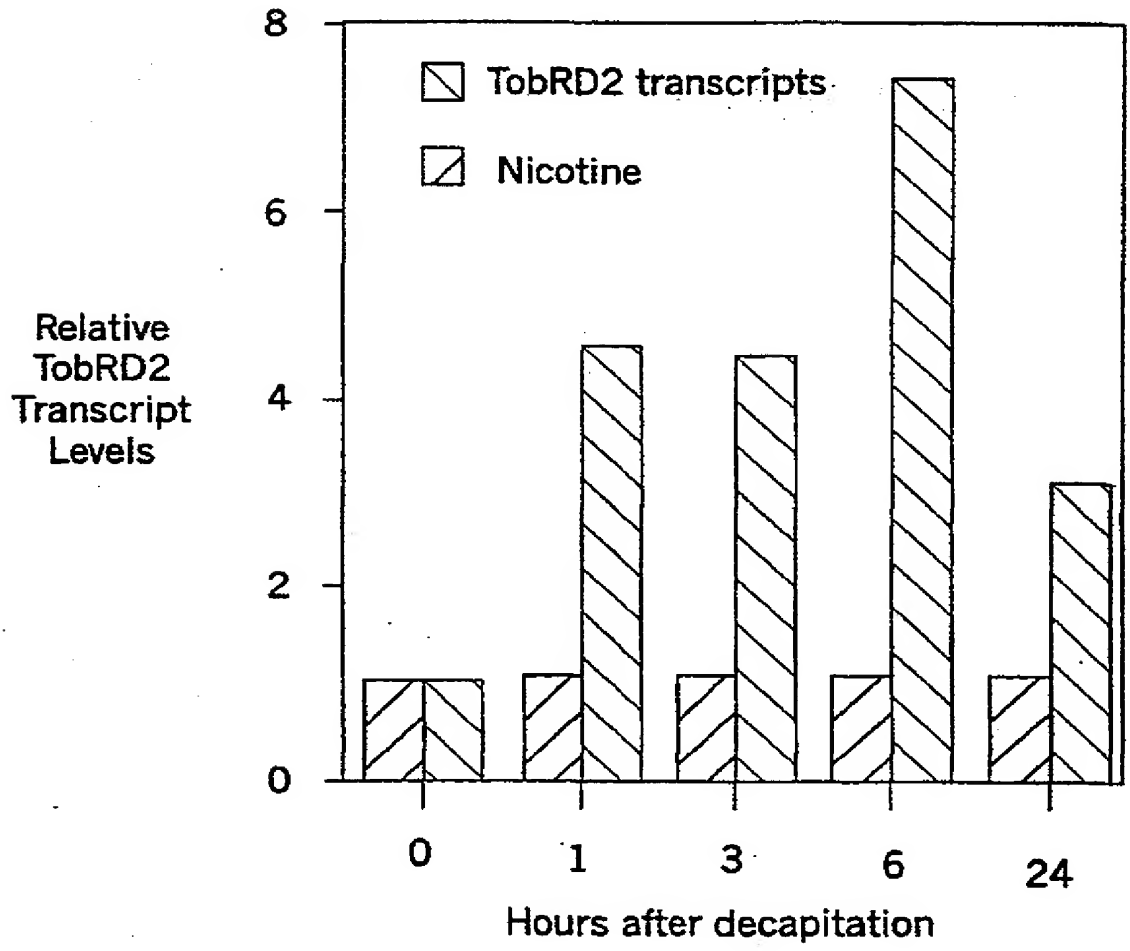

1.25
1
0.75
0.5
0.25
0
Relative Levels
TobRD2 transcripts
Nicotine
Nic1+ Nic2+
Nic1− Nic2+
Nic1+ Nic2−
Nic1− Nic2−

FIG. 6


8
6
4
2
0
TobRD2 transcripts
Nicotine
Relative TobRD2 Transcript Levels
0
1
3
6
24
Hours after decapitation